Experimental Studies of Mimicry. 7. Relative Palatability and Müllerian Mimicry among Neotropical Butterflies of the Subfamily Heliconiinae[1,2]

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(Plate I)

[This paper is a contribution from the William Beebe Tropical Research Station of the New York Zoological Society at Simla, Arima Valley, Trinidad, West Indies. The Station was founded in 1950 by the Zoological Society's Department of Tropical Research, under Dr. Beebe's direction. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of government forest reserves. The altitude of the research area is 500 to 1,800 feet, with an annual rainfall of more than 100 inches.

[For further ecological details of meteorology and biotic zones see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," by William Beebe, Zoologica, 1952, Vol. 37, No. 13, pp. 157-184].

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I. Introduction

THE choice of the Heliconiinae for this investigation is not without historical basis, for it was observations of these and other Neotropical butterflies that led naturalists in the latter half of the 19th century to formulate the theories known as Batesian and Müllerian mimicry. According to the theory of Bates (1862), rare palatable species, called the mimics, have gradually evolved through natural selection to resemble common unpalatable species, the models, of widely distinct taxonomic groups. This is brought about through the action of predators which, after trying a model insect, learn to associate its color-pattern with its noxious quality and so come to refuse it on sight. They then tend to confuse with the model and reject those naturally occurring variants in the palatable species which bear a resemblance to it. The early history and evidence for this phenomenon in many groups of insects and some vertebrates have been summarized by Carpenter & Ford (1933). Additional indirect support for the theory of Batesian mimicry has been accumulated over the years (Carpenter, 1920, 1949; Sheppard, 1959; Brower & Brower, 1962b), and it has been demonstrated in laboratory experiments for butterflies (Brower, J., 1958 a, b, c), artificial mimics (Mühlmann, 1934; Schmidt, 1958; Sexton, 1960; J. Brower, 1960) and flies (Mostler, 1935; Brower, Brower & Westcott, 1960; Brower & Brower, 1962a), with the use of toads, lizards and a variety of birds as caged predators. Further light has been shed upon the evolution of mimicry by the genetical studies of Ford (see review, 1953), Sheppard (1961,

1963), Clarke & Sheppard (1960, 1962) and Turner & Crane (1962).

However, it was clear even to Bates (1862) that resemblances also existed among species belonging to what he assumed to be one large distasteful family. Because his hypothesis required that mimicry occur between members of unrelated groups, he concluded that the likeness in these instances was due not to the adaptation of one to the other, but to the similar adaptation of all to the same local, probably inorganic, conditions. Wallace (1871) was also puzzled by the resemblances among related butterflies and suggested that the "distasteful secretion is not produced alike by all members of the family, and that where it is deficient, protective imitation comes into play" (p. 85). This statement was of the utmost importance because it initiated the idea of varying degrees of unpalatability within a group and in addition anticipated the line of reasoning later developed by Müller (1879). He, too, had had experience in the Neotropics, and from his observations on two butterflies, *Thyridia* and *Ituna,* arrived at a new hypothesis. Because of their similar appearance, these two genera had until that time been lumped in the family Danaidae. On several morphological bases, Müller separated them, leaving *Ituna* with the Danaidae but placing *Thyridia* with the Ithomiidae. He then realized that these two butterflies which superficially resembled each other both belonged to supposedly distasteful families. In addition species in the one genus sometimes outnumbered those in the other and *vice versa* in the natural environment. These facts did not meet the conditions of mimicry in the Batesian sense where one member of a similar pair is palatable and rare. Müller reasoned that if each predator has to learn the distinction between unpalatable and palatable species, then a certain number of individuals of both must fall victim to the inexperience of young enemies. But if two *unpalatable* species are sufficiently alike to be confused by predators, a lesson learned on one will also benefit the other. Thus the two will tend to converge upon a common color-pattern through selection by their insectivorous enemies, resulting in *Müllerian mimicry*.

The evolution of the convergence was at first misunderstood, as evidenced by the arguments which the Müllerian hypothesis generated over the selective advantage of mimicry to the more and less numerous members of an unpalatable complex. Marshall (1908) held that if species A outnumbered species B, B could evolve towards A but the reverse would not occur because any mutant of A that resembled B would be selected against by predators. Dixey (1908) countered this by saying that any mutant which was intermediate between A and B would gain the full advantage of both, and the two species would converge upon a mutual color-pattern. Fisher (1927, 1930 and 1958) pointed out the fallacies in both arguments by showing that while the less frequent species would derive the greater advantage, nevertheless the more abundant one would gain slightly by the pooled resemblance. The result would be a tendency for the two to converge upon a mutual color-pattern, but at unequal rates. Thus even a very rare unpalatable species does contribute to the overall effectiveness of Müllerian mimicry, is not detrimental to the common member, and should be considered a functional part of the complex. Fisher's clarification of this has been overlooked in some important papers on Müllerian mimicry (Darlington, 1938; Linsley, Eisner & Klots, 1961). Huheey (1961) has opened a new and very promising aspect of the problem in a discussion of the possible evolution of a Müllerian situation from a Batesian one.

But in spite of the voluminous literature on the natural history and the theoretical implications of Müllerian mimicry, very little experimental evidence has been produced, as was the case with Batesian mimicry until recently. The occurrence of potential models or Müllerian mimics in butterflies has been inferred the world over wherever their larvae eat the so-called poisonous foodplants, for example, those of the families Asclepiadaceae (milkweeds), Asaraceae (birthworts) and certain of the Solanaceae (nightshades). However, this correlation does not always hold, since species of the Passifloraceae (passion flowers) are foodplants of the heliconiines (Alexander, 1961a) and these are generally not cited as being poisonous (Muenscher, 1939). The early contributions discussing systematics and Müllerian mimicry in the heliconiines include Müller (1877), Dixey (1897), Stichel & Riffarth (1905), Kaye (1906, 1916), Moulton (1908), Seitz (1913) and Eltringham (1916). Numerous further instances commonly cited as Müllerian mimicry occur in the Hemiptera, Coleoptera and various families of moths. In these, there is a widespread repetition of orange or red and black coloration, together with the possession of noxious body fluids or defensive glands, and in the Hymenoptera there is, for example, the frequent occurrence of yellow and black circular banding associated with a stinging apparatus. (See Nicholson, 1927, and Linsley, 1959, for valuable summaries). Some experimental evidence for the unpalatability and mimicry of beetles of the family Lycidae has been obtained by Carpenter (1921) in Africa

with monkeys, *Cercopithecus* sp., as predators, by Darlington (1938) in Cuba with lizards, *Anolis sagrei* (Duméril & Bibron), and by Linsley, Eisner & Klots (1961) in Arizona with a variety of vertebrates and invertebrates. The only evidence on palatability for the heliconiines ("Heliconii") known to us is that obtained by Belt (1874, p. 242). He noted that a pair of birds bringing butterflies and dragonflies to their young never included these "Heliconii" which were extremely common in the area and he also showed that they were unpalatable to a tame White-faced Monkey (*Cebus* sp.).

The purpose of this paper is to present the results of an experiment designed to ask the following basic questions about Müllerian mimicry in the classical heliconiine butterflies: (A), Are these insects unpalatable to bird predators? (B), Do differences in unpalatability exist? (C), If so, do they correspond to the phylogenetic relationships of the butterflies? (D), Are heliconiine butterflies which resemble each other effective Müllerian mimics? In addition the experiment provided unanticipated evidence bearing on two further important aspects of mimicry theory. Framed as questions, these are (E), Do wild-caught birds behave in the laboratory in such a way as to suggest prior experience with Müllerian mimics in nature? and (F), Do birds generalize with regard to color, pattern or shape of the butterflies by transferring their learned rejection of a model to another heliconiine unlike the model-mimic pair?

II. Acknowledgments

III. Materials and Methods

Eight species of Heliconiinae consisting of four visually similar pairs, *Heliconius erato hydara* Hewitson and *Heliconius melpomene euryades* Riffarth; *Heliconius numata ethilla* Godart and *Heliconius isabella isabella* (Cramer); *Heliconius doris doris* (Linnaeus) and *Heliconius sara thamar* Hübner; *Dryas iulia iulia* (Fabricius) and *Agraulis vanillae vanillae* (Linnaeus) were tested for relative palatability and Müllerian mimicry (Plate I). All (except *H. isabella*) were reared in the laboratory and only males were used as test insects. The adults were killed by deep-freezing after they had aged for two days in an out-door flight cage (or shortly after capture in the wild for *H. isabella*). They were then thawed for a few minutes and placed with the wings spread in an open position in individual cellophane or glassine envelopes and kept in the deep freezer until used. This was done to alleviate the uncertainties of obtaining sufficient quantities of the needed species at the time the experiments had to be conducted. The freezing method also assured that the specimens were fresh and in a uniform state of preservation. Miriam Rothschild (*in litt.*, April 19, 1963) has pointed out that acetylcholine, which is found concentrated in certain distasteful insects, is inactivated by freezing. According to her, "the function of acetylcholine is not understood but there can be no doubt I think that it plays some part in enhancing the effect of poisons or harmful substances." Whether or not it will be found in quantity in the heliconiines remains to be discovered, but it should be emphasized here that the comparative palatability findings presented in this paper, based on individual butterflies which had been frozen, may be subject to some revision in the light of future work in the field of palatability biochemistry.

Care was taken to prevent desiccation of the insects while kept in the deep-freeze; wet toweling was placed in the freezer box (beneath and on top of wire screening in such a way as not to make contact with the butterflies) and the box was sealed with Scotch masking tape. Some of the butterflies reared and frozen in the spring of 1961, were used in the summer of 1962, as well as in 1961; the rest used in 1962 were preserved in the spring or summer of 1962. Butterflies used as edibles (see below) belonged to the family Satyridae and included various species of the genus *Euptychia* which were reasonably uniform in size and appearance. Except for being wild-caught and including both sexes, they were otherwise processed and given to the birds in a manner identical to the heliconiines.

The Silverbeak Tanagers, *Ramphocelus carbo magnirostris* Lafresnaye, which served as individually caged predators, were obtained by netting with Japanese mist nets at Cumuto Village, Waller Field and the Arima Valley. Mature individuals of both sexes, and birds born in the spring preceding the summers' experiments, were used. According to Herklots (1961), the Silverbeak breeds from February to August with the peak in April. Some of the birds were therefore quite young. They are a common species in Trinidad, and were suggested for experimental use by Dr. David Snow of Oxford University who informed us that they are omnivorous. We have observed them eating berries and in the early morning they were also seen on the Station grounds pecking up insects that had been attracted by laboratory lights the previous night. On June 21, 1963, a male Silverbeak was seen by Jogie Ramlal to capture a red and black *Heliconius* (*H. erato* or *H. melpomene*) on the wing in the natural habitat at Waller Field.

Several birds at a time (up to 10) were used in an experiment, and a surplus was maintained in a storage cage for replacements after it was found that mortality among them was high. Towards the end of the second summer it was found that deaths could be reduced by putting freshly-captured individuals in a flight cage with others which had become cage-adapted.

The experimental bird cages were modified from our basic design used previously at the Archbold Biological Station, Florida (J. Brower, 1958a). Each was a 30-inch cube framed with galvanized steel and covered with ¼-inch galvanized wire mesh. The front was covered by a removable galvanized steel sheet into which was set at eye level a piece of one-way glass 8 inches by 8 inches. A small sliding door was present at the bottom center of the front. A 60-watt incandescent light was placed over the center

of each cage. In this way the light was such that the inside could be seen by an observer looking in through the glass, but a bird occupying the cage could not see out. This helped to shorten the time taken for a bird to adapt and lessened distractions in the environment during an experiment. Each cage was equipped with a sand-covered floor, a water tube and a basic diet of commercial dog food, corn meal and banana mashed together plus one-half of a banana sliced longitudinally. This food was removed from the birds' cages at 6:50 a.m., the experiments were conducted from 8:00—11:30 a.m. and the food was replaced thereafter. The ten cages were in a roofed, large, outdoor cage covered with screening to prevent individuals from accidentally escaping during transfer. The sand was cleaned approximately once a week by sifting.

The butterflies were presented to the birds with 12-inch-long forceps. The sliding door was opened, the butterfly placed on the center of the sand floor, dorsal side up with wings outspread, the forceps were removed, and the door was shut. Each bird was allowed two minutes, timed with a stopwatch (or sweep-second wristwatch) to respond to each butterfly. The birds characteristically ate the *Euptychia* edibles by pecking them up and swallowing them whole. Butterflies not touched or dismembered were removed from the cage immediately after the two-minute period. Each bird was given at least 12 hours to become familiar with its own cage and was required to eat five or more *Euptychia* edibles in a one morning period before qualifying for the experiment. A large number of birds failed this initial test, even though worked with for several consecutive mornings.

IV. Experimental Design

Experimental procedure was as follows (see also Table 1 and Plate I). Individuals of one of eight species of heliconiines, consisting of four pairs with both members in each similar in size, shape and color-pattern, were offered one at a time to the singly-caged bird predators. Twenty males of one heliconiine species were given to a bird along with twenty satyrid edibles. The sequence of presentation of these models and edibles was determined randomly by pairs, as in earlier experiments (J. Brower, 1958a). In this way, no bird could learn on the basis of the order of presentation whether the next butterfly would be an edible or a model. Three to five trials (six to ten butterflies in total) were offered to a bird per day. As soon as a bird had been given the 20 models, the presumed Müllerian mimic was substituted for the model for five trials to

test for mimicry. Initially it was thought that considerable variation in the treatment of the mimics would occur and for this reason five mimics were given to allow for variance anaylsis. However, the results were so consistent that the treatment of only the first of the five mimics is analyzed in this paper (see Table 5). Finally a single other heliconiine butterfly, which will be called the "generalization butterfly," was offered to the bird. This differed in color and pattern from the model-mimic pair (Plate I) and was given to clarify the basis of rejection of the mimic heliconiine, that is, whether the bird would confuse only two very similar heliconiines, or whether it would generalize and reject any heliconiine butterfly regardless of its degree of resemblance to the model.

It was planned to run a series of 10 birds simultaneously with several different species of heliconiines. All birds in any one series were to receive the same random sequence. At the completion of one series of birds, a second would be initiated, with all receiving a second random

TABLE 1. EXPERIMENTAL PROCEDURE

Example of one random sequence of presentation of a heliconiine model, its respective Müllerian mimic, standard edible insects (*Euptychia* spp.), and the generalization heliconiine offered one at a time to individually caged Silverbeak Tanagers.

1a	Edible	
b	Model	
2a	Edible	
b	Model	
3a	Model	
b	Edible	
4a	Edible	
b	Model	PALATABILITY TEST
•		
•		
•		
•		
•		
20a	Model	
b	Edible	
21a	Mimic	
b	Edible	
•		
•		
•		MIMICRY TEST*
•		
•		
25a	Mimic	
b	Edible	
26a	Generalization	
	Heliconiine	GENERALIZATION TEST
b	Edible	

*Five mimics were given but analysis is based solely on the first one (see text).

sequence. However, it was impossible to keep the birds in phase because some would eat more butterflies per day than others, some began to reject all edibles part way through the experiment and had to be eliminated, while others died before completing the run. Thus in practice several different random sequences were run simultaneously and out of phase. It was also planned to repeat the series several times so that results from a total of 10 birds for each of the 8 heliconiine species and its corresponding mimic could be compared. Table 2 shows the actual number of birds and butterflies tested, which is somewhat less than hoped for but nevertheless substantial. (It was not possible to investigate the palatability of *H. isabella*).

At the completion of the experiment the palatability of the butterflies was compared by statistically testing (F and t tests) the numbers of heliconiines not touched by the birds out of the 20 each was given, or less than 20 in the 9 instances where birds died or stopped eating edibles (Tables 3 and 4). With one exception (Table 3, superscript 6), birds which failed to complete less than 10 trials were disqualified so as to avoid biasing the data towards acceptability.

Müllerian mimicry was tested by a two-step chi square analysis, using the birds as their own controls. First, if generalized rejection of all heliconiine-like butterflies occurs after the birds learn not to touch the models through the series of trials, the number of birds which reject the mimic should be greater than the number of the same birds which previously rejected their first model (Tables 5, 6a). Second, if detailed Müllerian mimicry is operative, the number of birds which reject the mimic should be higher than the number of the same birds which reject the generalization butterfly (Tables 5, 6b).

V. RESULTS AND DISCUSSION

(A). *General Unpalatability of Heliconiine Butterflies*

The data in Table 2 confirm the prediction made at the outset of the experiment that heliconiine butterflies are generally unpalatable insects to avian predators. Whereas the Silverbeaks ate all the satyrid butterflies, they accepted only three heliconiine species as food and these only to a slight extent, the maximum number eaten being one-fourth of both *Dryas iulia* and *Agraulis vanillae.* The only other species they ate were *Heliconius doris* and *H. melpomene,* and these only 11% and 1% of the time, respectively. Moreover, an examination of the peck and kill categories, added together in Table 2, shows that the birds as a group were remarkably uniform

TABLE 2. MEAN REACTIONS OF 62 SINGLY CAGED SILVERBEAK TANAGERS TO 1,177 INDIVIDUALS OF 7 SPECIES OF NEOTROPICAL HELICONIINE BUTTERFLIES AS MODELS.

Butterfly Species	Relative Frequencies of Reactions				Numbers of	
	Not Touch	Peck	Kill	Eat	Butterflies	Birds
Heliconius numata	.91	.08	.01	.00	136	7
Heliconius melpomene	.90	.08	.01	.01	167	9
Heliconius erato	.82	.12	.06	.00	175	10
Heliconius sara	.82	.10	.08	.00	160	8
Heliconius doris	.71	.11	.07	.11	180	9
Dryas iulia	.62	.07	.06	.25	201	11
Agraulis vanillae	.58	.08	.10	.25	158	8
				Totals	1,177	62

in their behavior towards the butterflies. Either they learned not to take them in a few trials, ranging from 9% of the total trials for *H. numata* and *H. melpomene* to 13% for *D. iulia*, and to 18% for *H. erato, H. sara, H. doris* and *A. vanillae,* or they found them palatable and ate them. In other words, if the birds found the insects unpalatable, they rapidly associated this with their appearance and learned to reject them on sight after an average of between approximately 1.8 and 3.6 trials.

(B) *Specific Differences in Palatability*

In Table 3 the heliconiine species are arranged in order of increasing acceptability from left to right, as shown by the individual and mean relative frequencies of butterflies not touched by the birds. The differences are analyzed statistically in Table 4 and the logic of the

analyses will now be presented. Dixon & Massey (1957) was consulted for statistical procedures, and the .05 level of formal significance was chosen.

Examination of the data for individual birds as well as the variances for each group of birds shows that their treatment of the last three species was considerably more variable than that of the first four, which they more consistently rejected. Bartlett's test for the homogeneity of the seven variances shows lack thereof (P < .001, Table 4a), indicating that the difference in variability of the birds' behavior towards *H. numata, H. melpomene, H. erato* and *H. sara* as one group and *H. doris, D. iulia* and *A. vanillae* as a second group is real. This grouping is legitimate for two reasons. First, the variances within each group are homogeneous (.50 > P >.25 for both, Table 4a). Secondly, while the

TABLE 3. RANKED RELATIVE FREQUENCIES, MEANS, AND VARIANCES OF HELICONIINE BUTTERFLIES NOT TOUCHED AS MODELS BY 62 SINGLY CAGED SILVERBEAK TANAGERS* For statistical analyses, see Table 4.

	Heliconius numata	*Heliconius melpomene*	*Heliconius erato*	*Heliconius sara*	*Heliconius doris*	*Dryas iulia*	*Agraulis vanillae*
	.95	.95	1.00[6]	.95	.95	.95	.90
	.95	.95	.85	.90	.90	.95	.83[18]
	.95	.95	.85	.90	.85	.95	.80
	.94[16]	.90	.85	.85	.85	.90	.80
	.90	.90	.85	.80	.85	.80	.75
	.85	.88[17]	.80[10]	.75	.85	.75	.25
	.85	.85	.80[19]	.75	.55	.73[11]	.20
		.85	.80	.65	.50	.60[10]	.10
		.80[10]	.79		.10	.15	
			.75			.10	
						.00	
Means**	.91	.90	.82	.82	.71	.62	.58
Variances	.003	.004	.004	.010	.078	.135	.110
No. Birds	7	9	10	8	9	11	8

*Superscript figures represent total heliconiine models given to the bird when not 20, due to bird's death or failure to continue eating edibles.

**Means are calculated from No. Not Touched ÷ No. Given for all birds as in Table 2, which explains discrepancy of .01 for *H. melpomene, H. erato* and *D. iulia* if means are calculated from individual frequency values in this table.

larger variance of *H. sara* compared to the others in its group might suggest that it belongs to the second group, Bartlett's test shows that it is illegitimate to include it therein (P < .025, Table 4a). Thus on the basis of variability of treatment, the three species of butterflies in the second group appear to be more palatable than the four in the first.

The F-test applied to the first four species indicates that the differences of the means do not quite reach significance at the .05 level (Table 4b). The reason for this can be seen by examining the data and comparing the means of the four species by pairs with the t-test. These four themselves break into two subgroups. *H. erato* and *H. sara* were both rejected with a mean frequency of .82, while *H. numata* and *H. melpomene* were rejected with a mean frequency of .91 and .90, respectively. Comparisons between members of these two groups are all statistically significant, P varying from less than .005 to less than .05 (Table 4c). From this it can be concluded that *H. numata* and *H melpomene* are nearly alike in palatability, but less acceptable than the similar *H. erato* and *H. sara*.

The F-test for the last three species indicates that the means do not differ significantly from each other (.50 > P > .25, Table 4b). This is due to the large variances in the treatment of these three species by the birds. The indicated trend of increasing palatability from *H. doris* to *A. vanillae* will almost certainly prove to be significant when more data are available.

TABLE 4. STATISTICAL ANALYSES OF DATA IN TABLE 3

a. Bartlett's test for homogeneity of variances.
  1. All seven species:
     F = 8.900   d.f. = 6/3200   P < .001
  2. *H. numata, H. melpomene, H. erato*, and *H. sara:*
     F = .633   d.f. = 3/1667 .50 > P > .25
  3. *H. doris, D. iulia*, and *A. vanillae:*
     F = .336   d.f. = 2/1333 .50 > P > .25
  4. *H. sara, H. doris, D. iulia*, and *A. vanillae:*
     F = 3.276   d.f. = 3/1667 .025 > P > .01

b. Variance analyses of groups of means (F-test).
  1. *H. numata, H. melpomene, H. erato*, and *H. sara:*
     F = 2.66   d.f. = 3/30   .10 > P > .05
  2. *H. doris, D. iulia*, and *A. vanillae:*
     F = .318   d.f. = 2/25   .50 > P > .25

c. Variance analyses of means by pairs within groups having homogeneous variances (t-test).

	t	d.f.	P
H. numata vs. *H. melpomene*		(not significant by inspection)	
H. erato vs. *H. sara*		(not significant by inspection)	
H. numata vs. *H. erato*	3.00	15	P < .005
H. numata vs. *H. sara*	2.14	13	.05 > P > .025
H. melpomene vs. *H. erato*	2.76	17	P < .01
H. melpomene vs. *H. sara*	2.00	15	.05 > P > .025
H. doris vs. *A. vanillae*	.88	15	.90 > P > .80

d. Variance analyses of means by pairs between groups not having homogeneous variances (modified t-test, see text).

	t	d.f.	P
H. numata vs. *H. doris*	2.06	9	.05 > P > .025
H. numata vs. *D. iulia*	2.59	11	.025 > P > .01
H. numata vs. *A. vanillae*	2.77	7	.025 > P > .01
H. melpomene vs. *H. doris*	2.00	9	.05 > P > .025
H. melpomene vs. *D. iulia*	2.37	11	.025 > P > .01
H. melpomene vs. *A. vanillae*	2.69	7	.025 > P > .01
H. erato vs. *H. doris*	1.15	9	.20 > P > .10
H. erato vs. *D. iulia*	1.79	11	.10 > P > .05
H. erato vs. *A. vanillae*	2.02	7	.05 > P > .025
H. sara vs. *H. doris*	.9	10	.20 > P > .10
H. sara vs. *D. iulia*	1.75	13	.10 > P > .05
H. sara vs. *A. vanillae*	1.95	8	.05 > P > .025

Since the variances of the first four and last three differ so greatly, it is necessary to employ a modified t-test (Bennett & Franklin, 1954) to compare mean treatments by pairs between the two groups. When this is done, it is seen that with the exception of *H. erato* and *H. sara,* which do not differ significantly from *H. doris* or *D. iulia,* all other paired comparisons are significant below the .05 level (Table 4d).

From this analysis, the general conclusion is that the heliconiines do differ in their palatability. On the basis of the more variable treatment and the lower mean numbers rejected of the last three species, this group as a whole is more palatable than the group including *H. numata, H. melpomene, H. erato* and *H. sara.* Within these four, it is clear that *H. melpomene* and *H. numata* are less palatable than *H. erato* and *H. sara.*

(C). *Phylogenetic Relationships and Palatability*

It has long been known that the Lepidoptera represent a unique biological system for the measurement of evolutionary forces in nature. Bates (1863, P. 353) pointed this out graphically when he said nature writes on their wings "as on a tablet the story of the modifications of species, so truly do all changes of organization register themselves thereon." Goldschmidt (1945) presented a review of the ontogenetic development of pigment patterns in the Lepidoptera which summarized the facts and clarified the physiological basis of Bates' speculations. Briefly, the sequence of events that results in the development of pattern and color in the wings of these holometabolous insects does not begin until very late in the pupa, when the individual has nearly completed its transformation to the adult stage. If a mutation occurs which affects a process in the early part of this sequence, the resultant phenotype will be strikingly different, and conversely if it occurs later, the change in appearance will be correspondingly less. The most important functional consequence of this ontogenetic system is that both small and large changes in color-pattern can occur whose effect on other physiological processes of the individual is but slight. In other words, centripetal selection (Haldane, 1959) against genes which affect the color-pattern is weak because their adverse pleiotropic effects on the physiology of the individual are small, or in Wright's (1932) terminology, the valleys between adaptive peaks are shallow (Dobzhansky, 1951). The result of this is seen in the vast evolutionary diversification of color-pattern in the Lepidoptera and even more dramatically by the changes that can occur very rapidly in natural populations as actually observed in the instances of breakdown of mimetic pattern in the African nymphaline butterfly, *Pseudacraea eurytus* (Linnaeus) (Carpenter, 1920, 1949; Sheppard, 1959); and the modifications in color form of another nymphaline, *Melitaea aurinia* Rott. (Ford & Ford, 1930).

Generally speaking, as Bates (1863) also emphasized, the color patterns of the wings are indicative of phylogenetic relationships in the Lepidoptera. But one of the outstanding facts of mimicry is that this principle is violated because selection has favored the similarity of appearance between forms whether they are related, as is often the case in Müllerian mimicry, or not related as is always true in Batesian mimicry. In Müllerian situations, it is important to consider the difficulty alluded to by Fisher (1958, p. 173), of deciding whether the resemblance of two unpalatable species which are congeneric is the result of (1) convergent evolution in appearance due to Müllerian advantage, (2) parallel evolution, or lack of divergence, in appearance due to Müllerian advantage, or (3) parallel evolution, or lack of divergence, in appearance without Müllerian advantage being involved. Darlington (1938, P. 686) struggled with this problem in lycid beetles when he said, "The great similarity of the three Cuban species of *Thonalmus* may possibly be an example of Müllerian mimicry, but on the other hand, it may be due merely to their close relationship." As such, he considered only the first and third alternatives. The second possibility apparently did not occur to him, namely, that the beetles may have derived a Müllerian advantage with the result that any mutations causing a divergence in their appearance were prevented from becoming established. The point to be emphasized is that close affinity does not preclude Müllerian relationships since the selective process in preventing divergence can be basically the same as that bringing about convergence of widely different unpalatable organisms. Fox (1956, p. 10) also failed to appreciate the three possible alternatives in his revision of the Ithomiidae. He noted that pairs of distantly related species which are congeneric, or even those in different genera, occasionally are so similar that it is impossible to tell them apart by superficial examination, and said, "These are cases of parallel evolution, and whether 'mimicry' causes them or not I cannot say, but I am doubtful that it does." Once Müllerian advantage is demonstrated experimentally in any given instance, the third possibility becomes less probable. The choice between the first and second alternatives then rests on the determination of the phylo-

genetic relationships by taxonomic characters more conservative than color-pattern, *i.e.,* those characters on which the forces of centripetal selection resist change to a greater degree. Classically, these are the external morphological characters of eggs, larvae, pupae and adults, and the characters of the adult genitalia, but they should also include behavioral patterns, as pointed out by Crane and her co-worker for the Heliconiinae (Crane, 1957; Alexander, 1961a, b) as well as in the reviews of Hinde & Tinbergen (1958) and Mayr (1958) for animals in general. The study of differences in palatability among related species should also provide valuable evidence for their phylogenetic relationships. Although not emphasized previously, this is to be expected because the evolution of unpalatability may be autotoxic, which requires major biochemical readjustments (Roth & Eisner, 1962) that are bound to proceed at a rate slower than change in color. Phylogenetic conclusions based on comparative studies of morphology, behavior and palatability may therefore be expected to produce a considerable degree of concordance.

As shown by the present experimental study, the Heliconiinae, long assumed to be unpalatable to birds, are in fact so. These butterflies are a specialized subfamily of the large and diverse family Nymphalidae (Michener, 1942). On the basis of a large number of feeding experiments (Carpenter, 1921; Jones, 1932; Marshall, 1902, Pocock, 1911; Swynnerton, 1919) and extensively developed cryptic coloration (Cott, 1957), it is clear that the more primitive Nymphalidae (the subfamily Nymphalinae) are generally tasteful insects to avian predators, although their palatability characteristics should be considered relative rather than absolute, as discussed in theory by Fisher (1927, 1930, 1958), Nicholson (1927) and Carpenter & Ford (1933), and demonstrated by J. Brower (1958a, b, c). From these considerations it was predicted at the outset of the experiment that the heliconiine species phylogenetically closest to the Nymphalinae would be the most palatable. Detailed morphological studies of the immature stages of the Trinidad species by Fleming (1960) and Beebe, Crane & Fleming (1960) indicated that *Agraulis vanillae* and *Dryas iulia,* and also probably *Heliconius doris,* are closest to the nymphaline stock of the seven species tested in the present study. The more limited conclusions of Alexander (1961a, b), based on the comparative behavior of the immature stages, also agreed with this. It is therefore of the greatest interest that these three species were in fact found to be the most palatable (Tables 2, 3 and 4, and see above).

Crane and her co-workers have also given evidence that *H. numata, H. melpomene* and *H. erato* form a close group within which the former two species are more closely related than either is to *H. erato,* in spite of the fact that *H. melpomene* and *H. erato* are nearly indistinguishable as adults. The palatability data statistically confirm this since the birds treated *H. numata* and *H. melpomene* in a similar manner whereas their treatment of both of these differs significantly from *H. erato* (P < .005, P < .01, respectively, Table 4c). The birds' treatment of *H. erato* and *H. sara* is also consistent with their scheme. Thus the palatability characteristics of the adult male heliconiines in this study are remarkably concordant with the phylogenetic conclusions based on morphology and behavior.

Now that the relationships of *H. numata, H. melpomene* and *H. erato* have been partially elucidated, it would be of the greatest interest to initiate a program of hybridization in an attempt to reconstruct further the evolutionary changes that led, for example, to the convergence in color and pattern of *H. melpomene* and *H. erato,* or as another alternative, to the divergence of *H. numata* from the *melpomene-erato* color-pattern through its convergence with the *H. isabella* color-pattern.

(D). *Müllerian Mimicry*

It may be seen in Table 5 that 21 out of 62 (34%) of the birds did not touch the first model given, although all but one subsequently pecked, killed or ate at least one model in their individual series of 20. Taking these initial rejections of models as a base line for comparison, we should expect that the birds would not touch a higher proportion of mimics after experiencing the series of 20 models if mimicry is effective. Of the 62 birds which began the experiment, 10 died and 8 either found the heliconiines palatable or failed to learn to avoid them on sight, leaving a total of 44 birds. Of these, 42 (95%) did not touch their first mimics (Table 5). In other words, 61% more of the birds rejected their mimics than their first models. This difference is highly significant (P < .001, Table 6a).

It can be seen in Plate I that the difference in appearance between the satyrid edibles and the heliconiine models and mimics is greater in size, shape, contrast and brightness of color than among the heliconiines themselves. The possibility therefore existed in the experiment that the birds, having discovered the difference in palatability between the two, would form a generalized rejection response towards all heliconiine-like butterflies. If this were true, it would be expected that the birds which learned to reject

TABLE 5. COMPARISON OF THE NUMBER AND FREQUENCY OF BIRDS WHICH REJECTED THEIR FIRST
MODELS COMPARED TO THE SAME BIRDS WHICH SUBSEQUENTLY REJECTED THEIR FIRST MIMICS
AND GENERALIZATION BUTTERFLIES AFTER HAVING EXPERIENCED 20 MODELS
For statistical analyses, see Tables 6-9.

Models	Mimics	Generalization Butterflies	Numbers and Frequencies of Birds Not Touching		
			First Model	First Mimic	Generalization Butterfly
H. numata	*H. isabella*	$\begin{cases} doris \\ vanillae \end{cases}$	4/7 (.57)	5/6 (.83)	$\begin{cases} 1/2 \ (.50) \\ 2/4 \ (.50) \end{cases}$
H. melpomene	*H. erato*	*vanillae*	4/9 (.44)	7/7 (1.00)	3/7 (.43)
H. erato	*H. melpomene*	*vanillae*	5/10 (.50)	6/6 (1.00)	2/5 (.40)
H. sara	*H. doris*	*vanillae*	0/8 (.00)	7/8 (.88)	6/8 (.75)
H. doris	*H. sara*	*vanillae*	3/9 (.33)	7/7 (1.00)	1/7 (.14)
D. iulia	*A. vanillae*	*doris*	3/11 (.27)	6/6 (1.00)	6/6 (1.00)
A. vanillae	*D. iulia*	*doris*	2/8 (.25)	4/4 (1.00)	4/4 (1.00)
		Totals	21/62* (.34)	42/44* (.95)	25/43** (.58)

*Discrepancy of 18 birds due to the death of 10, plus 8 which did not learn to reject models before being given mimics.

**Discrepancy of 1 bird due to death prior to being given the generalization butterfly.

the models would reject their mimics and generalization heliconiines to approximately the same extent. This they did not do; 95% of them in fact rejected the mimics as compared to 58% for the generalization insects (Table 5). This difference of 37% greater protection for the mimics is highly significant (P < .001, Table 6b). Since it has been shown that all seven of the heliconiine butterflies studied were distasteful, it is concluded that detailed Müllerian mimicry is highly effective under the conditions of the experiment. Moreover, an examination of the data for individual birds in Table 5 shows that they were extremely consistent in reducing their attacks on the mimics; in no model-mimic pair was the percent not touching the mimics less than that not touching first models. Reasons why a considerable proportion of the birds rejected their first models and generalization heliconiines will be considered below.

(E). *Evidence that Müllerian Mimicry Operates in Nature*

1. *Treatment of First Models*

All the birds used in these experiments were captured in the wild and used in the tests within one week after being caught. In so far as the heliconiine butterflies studied are among the most abundant diurnal Lepidoptera in Trinidad, it seems likely that the birds may have had prior experience with them in nature. It has been shown above that all the birds, with a single exception, attacked at least one of the heliconiines given to them in the series of 20. However, one-third of the birds (21/62) rejected the initial model (Table 5). On the hypothesis

that these rejections were based partly on the birds' remembrance of a prior experience in the wild with the unpalatable butterflies, it is to be expected that the heliconiines most rejected initially would be those which are the commonest in the habitats where the birds occur, since it would be these with which the birds would most likely have come in contact. Now in the lower montane and savanna forest where the Silverbeak Tanagers breed and are abundant, without doubt the most common distasteful butterfly seen is *Heliconius erato*. Moreover, although *H. numata* itself is not generally abundant, there exists a whole assemblage of classical Müllerian mimics similar to it in color and pattern which fly together. These include not only *H. numata* and *H. isabella,* but at least seven species belonging to the Ithomiidae and also *Lycorea ceres ceres* (Cramer), a member of the Danaidae. Assuming that these other species are in fact unpalatable, as the heliconiines are, we can then say that the birds should, when brought into the laboratory and tested, reject a higher proportion of *H. numata, H. isabella, H. erato* and the latter's very close Müllerian mimic, *H. melpomene,* than the other four species. The data in Table 7a, extracted from Table 5, fully confirm this expectation (except for *H. isabella,* for which palatability tests were not made). Thus 50% of the birds as a group did not touch the initial models when these were *H. numata, H. melpomene* and *H. erato,* whereas only 22% of them rejected the initial individuals of *H. sara, H. doris, D. iulia* and *A. vanillae.* This difference is statistically significant (P < .05).

TABLE 6a. STATISTICAL ANALYSIS OF THE REACTIONS OF ALL BIRDS TO THEIR FIRST MODEL *vs.* THE SAME BIRDS' REACTIONS TO THEIR FIRST MIMIC, SHOWING THAT MÜLLERIAN MIMICRY IS HIGHLY EFFECTIVE EITHER THROUGH DETAILED MIMICRY OR THROUGH GENERALIZED REJECTION OF ALL HELICONIINE-LIKE BUTTERFLIES.
(Data from Table 5).

Category of Reaction	Reaction of Birds to		
	First Model	First Mimic	Totals
Not Touch	21 (34%)	42 (95%)	63
Peck, Kill, or Eat	41	2	43
Totals	62	44	106

Exact chi square = 37.74; d.f. = 1; P < .001

TABLE 6b. STATISTICAL ANALYSIS OF THE REACTIONS OF ALL BIRDS TO THEIR FIRST MIMIC *vs.* THE SAME BIRDS' REACTIONS TO THEIR GENERALIZATION BUTTERFLY, SHOWING THAT DETAILED MIMICRY CONFERS PROTECTION BEYOND THAT RESULTING FROM GENERALIZATION.
(Data from Table 5).

Category of Reaction	Reaction of Birds to		
	First Mimic	Generalization Butterfly	Totals
Not Touch	42 (95%)	25 (58%)	67
Peck, Kill, or Eat	2	18	20
Totals	44	43	87

Exact chi square = 15.06; d.f. = 1; P < .001

An alternative explanation for the greater initial rejection of *H. numata, H. melpomene* and *H. erato* is that the birds had tried all seven species in nature, but had remembered their experience to a greater extent for these three species because of their demonstrated high unpalatability. This explanation seems the less likely because, if it were correct, then *H. sara* should have been rejected initially to an extent as great as *H. erato* whose palatability characteristics it shares (Tables 3 and 4), and it clearly was not (Table 5). Moreover, if palatability were the basis, there should be some tendency for the birds to reject *H. sara* and *H. doris* initially to a greater extent than *D. iulia* and *A. vanillae*. This is definitely not so, as shown statistically in Table 7b. However, no matter which of these explanations is correct, both are based on prior experience. Therefore, the hypothesis is supported that these birds actually were involved as selective agents for the evolution of Müllerian mimicry in Trinidad when they were captured.

Two other potential explanations exist which are not based on prior experience. It might be argued that the rejection of more of these first three species could be the result of a size differ-

ential between them and the last four. This, however, is excluded because the size range of the former group is included completely within that of the latter (see Plate I). Secondly, the birds might have an innate tendency to reject them. This is unlikely for several reasons. First of all, one of the characteristics of innate behavior is its consistency, and if operating here we should expect the birds to behave uniformly towards the model species, either all rejecting or all accepting them. Considering the treatment of first models again, we see that only in the instance of *H. sara* were the birds uniform (Table 5), and on the average 34% attacked them, which is clearly neither consistent rejection nor acceptance. Furthermore, all the birds except one attacked at least one model in the series of 20. Finally, as will be discussed in a future publication, innate compared to learned rejection responses towards potential food items bearing the kinds of patterns that are involved in mimicry are highly inefficient from an ecological viewpoint in animals that have a reasonable capacity to learn. Briefly, this is because in Batesian mimicry the predators would be deprived of a valuable food supply should the mimic species come greatly to outnumber the model.

TABLE 7a. STATISTICAL ANALYSIS OF THE REACTIONS OF BIRDS TO THEIR FIRST MODEL, COMPARING *H. numata, H. melpomene* AND *H. erato* vs. *H. sara, H. doris, D. iulia* AND *A. vanillae*. SEE TEXT FOR INTERPRETATION. (Data from Table 5).

Category of Reaction	Reaction of Birds to First Model of the Species		
	n., m., e.	s., d., i., v.	Totals
Not Touch	13 (50%)	8 (22%)	21
Peck, Kill, or Eat	13	28	41
Totals	26	36	62

Exact chi square = 4.03; d.f. = 1; .05 > P > .025

TABLE 7b. STATISTICAL ANALYSIS OF THE REACTIONS OF BIRDS TO THEIR FIRST MODEL, COMPARING *H. sara* AND *H. doris* vs. *D. iulia* AND *A. vanillae*. SEE TEXT FOR INTERPRETATION. (Data from Table 5).

Category of Reaction	Reaction of Birds to First Model of the Species		
	s., d.	i., v.	Totals
Not Touch	3 (18%)	5 (26%)	8
Peck, Kill, or Eat	14	14	28
Totals	17	19	36

Exact chi square = .05; d.f. = 1; P > .20

This is bound to occur occasionally as animals typically fluctuate in abundance (Lack, 1954). Moreover, because palatability is a relative phenomenon, as discussed above, depending among other things on the degree of the predator's hunger, to be predisposed never to take warningly-colored insects and those involved in Müllerian mimicry seems similarly inefficient. Thus on the basis of the data presented in this paper and from more general considerations, the hypothesis of innate rejection of these heliconiine butterflies is discarded (see also Brower & Brower, 1962a, where similar findings were obtained with toads as experimental predators of bees and mimetic flies).

2. Treatment of Generalization Butterflies

The birds' responses to the generalization butterflies, *H. doris* and *A. vanillae*, compared to their treatment of the first models, as well as a comparison between the two generalization species, provide an additional line of evidence that they had had prior experience with these heliconiines in nature.

In Table 8a, it can be seen that the birds as a group rejected the generalization butterflies to a greater extent (58%) than the first models (34%). This difference of 24% is statistically significant (P < .025). Tables 8b and 8c break down Table 8a and show that the significance is due to the birds' rejection of *H. doris* (Table 8b)

as the generalization butterfly, but not *A. vanillae* (Table 8c), and Table 8d shows that the difference between *H. doris* and *A. vanillae* is indeed significant (P < .025). In other words, after the birds received their series of distasteful *H. numata, H. melpomene, H. erato, H. sara* and *H. doris,* they did not reject *A. vanillae* as the generalization insect (Table 8c); but after their series of distasteful *A. vanillae* and *D. iulia,* they did reject *H. doris* as the generalization insect (Table 8b). The most probable explanation of the treatment of *A. vanillae* as the generalization insect is that the birds did not associate it with the bicolored *Heliconius* spp. The fact that the birds' rejections of *A. vanillae* and *D. iulia* as first models did not differ significantly from their rejections of *A. vanillae* when it was the generalization insect (P > .20, Table 9a) further supports this view. On the other hand, it seems most probable that the birds' rejection of *H. doris* as the generalization insect after their series of *A. vanillae* or *D. iulia* is based on the fact that distasteful monocolored heliconiines in some way recalled prior experience with *H. doris,* or *H. sara,* or with the bicolored *Heliconius* spp. in general which they had learned in nature were even more distasteful than *D. iulia* or *A. vanillae.* The fact that the birds' rejections of *H. doris* (and *H. sara*) as first models did differ significantly from their rejection of *H. doris* as the generalization insect (P < .001, Table 9b) supports this view.

TABLE 8a. STATISTICAL ANAYLSIS OF REACTIONS OF ALL BIRDS TO THEIR FIRST MODEL *vs.* THE SAME BIRDS' REACTIONS TO THEIR GENERALIZATION BUTTERFLY, SHOWING THAT THE BIRDS DO GENERALIZE. (Data from Table 5).

Category of Reaction	Reaction of Birds to		
	First Model	Generalization Butterfly	Totals
Not Touch	21 (34%)	25 (58%)	46
Peck, Kill, or Eat	41	18	59
Totals	62	43	105

Exact chi square = 5.13; d.f. = 1; .025 > P > .01

TABLE 8b. STATISTICAL ANAYLSIS OF REACTIONS OF BIRDS TO THEIR FIRST MODEL *vs.* THE SAME BIRDS' REACTIONS TO THEIR GENERALIZATION BUTTERFLY WHEN MODELS ARE *D. iulia* AND *A. vanillae* AND THE GENERALIZATION BUTTERFLY IS *H. doris*, SHOWING THAT BIRDS DO GENERALIZE TO *H. doris*. (Data from Table 5).

Category of Reaction	Reaction of Birds to		
	First Model (*i. & v.*)	Generalization Butterfly (*d.*)	Totals
Not Touch	5 (26%)	10 (100%)	15
Peck, Kill, or Eat	14	0	14
Totals	19	10	29

Exact chi square = 11.45; d.f. = 1; P < .001

TABLE 8c. STATISTICAL ANALYSIS OF REACTIONS OF BIRDS TO THEIR FIRST MODEL *vs.* THE SAME BIRDS' REACTIONS TO THEIR GENERALIZATION BUTTERFLY WHEN MODELS ARE *H. numata, H. melpomene, H. erato, H. sara* AND *H. doris* AND THE GENERALIZATION BUTTERFLY IS *Agraulis vanillae*, SHOWING THAT BIRDS DO NOT GENERALIZE TO *A. vanillae*. (Data from Table 5).

Category of Reaction	Reaction of Birds to		
	First Model (*n., m., e., s., & d.*)	Generalization Butterfly (*v.*)	Totals
Not Touch	16 (37%)	14 (45%)	30
Peck, Kill, or Eat	27	17	44
Totals	43	31	74

Exact chi square = .20; d.f. = 1; P > .20

TABLE 8d. STATISTICAL ANALYSIS OF REACTIONS OF BIRDS TO THEIR GENERALIZATION BUTTERFLY, SHOWING GREATER GENERALIZATION TO *H. doris* THAN TO *A. vanillae*. (Data from Table 5).

Category of Reaction	Reaction of Birds to		
	Generalization Butterfly		Totals
	(*v.*)	(*d.*)	
Not Touch	14 (45%)	11 (92%)	25
Peck, Kill, or Eat	17	1	18
Totals	31	12	43

Exact chi square = 5.90; d.f. = 1; .025 > P > .01

TABLE 9a. STATISTICAL ANALYSIS OF REACTION OF BIRDS TO *A. vanillae* AND *D. iulia* AS FIRST MODELS*, COMPARED TO THE REACTIONS OF BIRDS TO *A. vanillae* AS THEIR GENERALIZATION BUTTERFLY AFTER EXPERIENCING *H. numata, H. melpomene, H. erato, H. sara* OR *H. doris* AS MODELS. SEE TEXT FOR INTERPRETATION. (Data from Table 5).

| | Reaction of Birds to | | |
Category of Reaction	First Model (*v. & i.*)	Generalization Butterfly (*v.*)	Totals
Not Touch	5 (26%)	14 (45%)	19
Peck, Kill, or Eat	14	17	31
Totals	19	31	50

Exact chi square = 1.07; d.f. = 1; P > .20

*Since the birds treated *A. vanillae* and *D. iulia* as Müllerian mimics, as well as *H. doris* and *H. sara*, it is legitimate to lump each pair as first models and consider the first as the "*vanillae* visual species" and the second as the "*doris* visual species."

TABLE 9b. STATISTICAL ANALYSIS OF REACTIONS OF BIRDS TO *H. doris* AND *H. sara* AS FIRST MODELS*, COMPARED TO THE REACTIONS OF BIRDS TO *H. doris* AS THEIR GENERALIZATION BUTTERFLY AFTER EXPERIENCING *A. vanillae* AND *D. iulia* AS MODELS. SEE TEXT FOR INTERPRETATION. (Data from Table 5).

| | Reaction of Birds to | | |
Category of Reaction	First Model (*d. & s.*)	Generalization Butterfly (*d.*)	Totals
Not Touch	3 (18%)	10 (100%)	13
Peck, Kill, or Eat	14	0	14
	17	10	27

Exact chi square = 13.96; d.f. = 1; P < .001

*Since the birds treated *A. vanillae* and *D. iulia* as Müllerian mimics, as well as *H. doris* and *H. sara*, it is legitimate to lump each pair as first models and consider the first as the "*vanillae* visual species" and the second as the "*doris* visual species."

(F). *Evidence that a Generalized Resemblance Confers a Mimetic Advantage*

If the species of the genus *Heliconius* (i.e., excluding *Agraulis* and *Dryas*) are carefully examined, it is clear to a human observer that they are similar in shape, body form and details of morphology such as wing venation, antennae and legs. Wallace (1871, p. 85) referred to this "uniformity of type with great diversity of colouring," and suggested that a predator would tend to recognize them as a unit of unpalatability. This statement was important because it alluded to the idea that a resemblance other than detailed similarity in color-pattern might be sufficient for a predator to associate the unpalatability of any one species with the others.

Some experimental evidence has indicated that bird predators do tend to generalize from an unpleasant experience. The work of Lloyd Morgan (1900) was the first to demonstrate this. He showed that young chicks which were fed qui-nine-treated meal on a striped glass slip associated the striped condition with the unpleasant food, because later they would not eat untreated meal from the banded slip. These same birds then refused to peck a striped Cinnabar caterpillar which they had never seen before. In another experiment he showed that chicks trained to reject Cinnabar larvae would not touch wasps which they had never experienced. Windecker's (1939) results confirmed the Cinnabar larva-wasp generalization. Mühlmann's (1934) study with artificial models and mimics (painted meal-worms) also showed generalization by the bird predators. In experiments with butterflies which were offered to caged Scrub Jays, *Aphelocoma c. coerulescens* (Linnaeus)[4], J. Brower (1958c) found that jays which had experienced the model *Danaus plexippus* (Linnaeus) later refused it

[4]Scrub Jays were previously referred to as *Cyanocitta c. coerulescens* (Bosc) (J. Brower, 1958a, b, c) after Amadon (1944).

and its mimic *Limenitis archippus archippus* (Cramer) on sight. Moreover, these birds also rejected on sight a different geographic race of the same species, *L. a. floridensis,* (Strecker) which mimics the much darker *Danaus gilippus berenice* (Cramer), and then even the latter. In addition, Schmidt (1958) found that domestic chicks learned to reject artificial models and then artificial mimics which were painted so as to be considerably different from the models in color-pattern.

  These experimental studies of generalization suggest that birds in nature are likely to treat incipient mimics conservatively. However, as Swynnerton (1915) was the first to emphasize, vertebrate predators which have associated the coloration of an insect with its distastefulness nevertheless characteristically make errors and attack the species again. Where incipient Müllerian mimicry is involved, the predator's mistake results in another unpleasant experience and so tends to lessen further attacks on both model and mimic at least temporarily. But even more important was Swynnerton's (1915) discovery that once a given warning pattern is learned, simply seeing the insects which bear the pattern, without attacking them, leads to a further reduction in the number of errors they make. The phenomenon in fact represents a general principle of animal behavior known as secondary reinforcement (Thorpe, 1956). In his independent discovery of this, Swynnerton deduced an important new principle of Müllerian mimicry, namely that the greater the numbers of unpalatable species and individuals which bear a common warning color-pattern, the fewer the mistaken attacks will be made. To take an extreme hypothetical example, if 100 attacks are made on 1,000 individuals, only 90 might be made on 2,000. It should be noted that the behavioral principle (secondary reinforcement) involved here results in a type of mathematical death rate relationship which population biologists recognize as "inversely density-dependent" (Holling, 1961), or in Nicholson's (1954) terminology, "density-disturbing" (see also Varley, 1957).

  In view of this, one might wonder why all sympatric members of the Heliconiinae do not share a single color-pattern. The answer to this appears to lie in the balance between the protective advantage of Müllerian mimicry, as opposed to mere warning coloration, and the courtship disadvantage that would result from interspecific interference among several sympatric species should they all bear the same visual cues (Poulton, 1907; L. Brower, 1959). Further work along these lines will possibly elucidate why certain of

the heliconiines are polymorphic, which, as Sheppard (1963) has pointed out, is contrary to prediction of Müllerian mimicry theory. On the other hand, it may well be that the large amount of convergence that has occurred in butterflies involved in Müllerian mimicry complexes (reaching its zenith in the "tiger-stripe" complex of the South American Ithomiidae, Heliconiinae and Lycoreinae; see Kaye, 1906, and Moulton, 1908) has been made possible by the greater importance of scent than sight stimuli in their courtship. Without exception, they all have elaborate odor-disseminating organs, which is not true of those species which are Batesian mimics. This is discussed in detail elsewhere (L. Brower, 1963).

  It is well to remember that errors reinforce the lesson of distastefulness only if the situation is Müllerian; in Batesian mimicry the predator may be rewarded for his mistake by an edible insect, and if the frequency of a mimic becomes high enough, the predator will begin to take it regularly, and mimicry will tend to become of much less advantage, as demonstrated directly by experiment (J. Brower, 1960) as well as indirectly, as reviewed by Brower & Brower (1962b) and Sheppard (1959). Fisher, (1958, p. 166) has concisely described this aspect of Batesian and Müllerian mimicry as follows: "The Batesian mimic gains its advantage at the expense of the predator which it deceives, and of the model whose life it endangers. In the Müllerian system both species alike are mimic and model, each reaps an advantage of the same kind, and both cooperate to confer an advantage upon the predator by simplifying its education. The predator which requires to frustrate the wiles of a Batesian mimic should develop a keen and sceptical discrimination; while he will best take advantage of the Müllerian situation by generalization, and reasoning from analogy." This last phrase appears to fit the behavior of the Silverbeak predators used in this experiment. The line of reasoning developed in the last section (E-2) showed that a series of experiences with distasteful monocolored orange butterflies *(A. vanillae* and *D. iulia)* caused the birds to reject bicolored blue and yellow ones *(H. doris)*. This finding, which suggests fascinating further research, is of the greatest importance to mimicry theory. It shows that a similarity in shape and size alone (compare *H. doris* to *D. iulia* and *A. vanillae* in Plate I) can result in a substantial selective advantage by provoking generalized rejection responses in birds which presumably have had considerable experience in the wild. As such, the data nullify experimentally for the first time with actual mimetic butterflies the argument of Punnet

(1915) and Goldschmidt (1945), who maintained that mimicry can not arise by the accumulation of small variations because the initial changes would not be at a selective advantage with regard to predators which had experienced the potential models. The contrary may now be stated as an experimentally demonstrated fact, namely, that even a remote resemblance between heliconiine butterflies can be advantageous.

VI. Summary

1. Seven species of heliconiine butterflies (*Heliconius numata, H. melpomene, H. erato, H. sara, H. doris, Dryas iulia* and *Agraulis vanillae*) were shown to be unpalatable to 62 individually caged passerine bird predators, the Silverbeak Tanager.

2. Differences in unpalatability exist among the species, and in general correspond to the phylogenetic relationships of the butterflies. This supports the prediction of mimicry theory that palatability evolves more slowly than color and pattern.

3. *Heliconius numata* and *H. melpomene* are probably more closely related to each other than either is to *H. erato*, even though *H. melpomene* and *H. erato* are nearly indistinguishable as adults (see Plate I).

4. Under controlled procedure in experimental cages, four pairs of heliconiine butterflies which resemble each other (see Plate I) are highly effective Müllerian mimics.

5. Evidence is presented that the birds used in these experiments had experienced heliconiines in nature and that Müllerian mimicry among these butterflies is conferring a protective advantage in Trinidad at the present time.

6. Data are presented which discard the hypothesis of innate rejection of warningly-colored heliconiines by the Silverbeak Tanager.

7. Evidence was found that even a generalized similarity in shape and size between two unpalatable species can result in a substantial selective advantage, thus nullifying the hypothesis of Punnet (1915) and Goldschmidt (1945).

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EXPLANATION OF THE PLATE

PLATE I

Representatives of the species of butterflies offered to caged Silverbeak birds in the experimental studies of relative palatability and Müllerian mimicry. The butterflies are arranged to elucidate the experimental procedure. From left to right, the vertical columns are: (1) various species of satyrids which served as standard edible insects (those illustrated here are related species from various parts of South and Central America, by courtesy of the American Museum of Natural History); (2) seven species of models; (3) their respective mimics; (4) the generalization butterflies (see text for explanation). Note that the specimen 5th from the top in the 4th column bears the beak-marks of a bird on its wings. The models, mimics, and generalization butterflies illustrated were captured in the wild in Trinidad during 1961 and 1962. Some of the heliconiine individuals are represented several times in the plate. (Approximately .4 natural size).